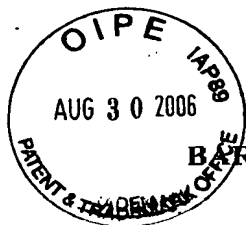


Exhibit A



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Group Art
Unit: 1642
Application No.: 10/626,905
Filing Date: July 25, 2003
First Named
Inventor: Franzoso, Guido, *et al.*
Title: *METHODS AND COMPOSITIONS
FOR MODULATING APOPTOSIS*
Attorney
Docket No.: 21459-94575
Examiner
Name: PHAM, AUDREY S.

Certificate Under 37 CFR 1.8(a)

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on Aug 30, 2006

Alice O. Martin
Alice O. Martin
Registration No. 35.601

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

1. I, Guido Franzoso, am a co-inventor of the patent application captioned above.
2. I have reviewed the patent application captioned above as filed, and the Office Action, mailed on March 30, 2006.
3. I understand that the examiner has rejected claims 1-2, and 6 for failing to teach one of skill in my art how to modulate the JNK pathway *in vivo*.

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4. I also understand the examiner doubts that I included *in vivo* methods in my invention.
5. I am submitting this Declaration to show that the specification as filed provides enabling disclosure to a person of ordinary skill in the art to practice the full scope of the disclosure. The data from Gadd45 β knock-out mice model in Exhibit A provide substantial evidence that Gadd45 β is a modulator of JNK pathway in regulating cell death *in vivo*.
6. Methods and compositions disclosed in the specification of my patent application enable a person of ordinary skill in the art to design and develop methods to modulate a pathway leading to programmed cell death *in vivo*.
7. On page 7 of the Office Action, the examiner stated that "neither the art nor the specification teaches a nexus between *in vitro* down regulating JNK pathway in culture cells and the *in vivo* efficacy of treating a variety of diseases". The examiner also questioned whether the *in vitro* results were artifacts of cell culture systems.
8. In the specification, FIGS. 19-20 show physical interaction between Gadd45 β and kinases in the JNK pathway in 293 cells. FIG. 21 shows Gadd45 β inhibits JNKK2 activity *in vitro*. FIG. 22A-B shows Gadd45 β inhibits JNKK2 activity in 3DO cells. FIG. 23-27 show that distinct polypeptide regions in JNKK2 and Gadd45 β interact. These figures and accompanying description in the specification demonstrate that Gadd45 β interacts with JNKK2 and further regulates JNKK2 activity. These data illustrate that Gadd45 β modulates JNK pathway by regulating JNKK2 activity.

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9. Following the guidance in the specification, we demonstrated that Gadd45 β modulates JNKK2 activity *in vivo*, in a mouse model, as shown in Exhibit A. Experiments involving Gadd45 β knock-out mice are shown in Exhibit A. Gadd45 β knock-out mice do not have functional Gadd45 β and therefore cannot modulate JNK activity. JNK activation leads to cell death in mice following hepatectomy.
10. Exhibit A demonstrates that the livers of Gadd45 β knock-out mice fail to regenerate following partial hepatectomy. Liver regenerative process is controlled by complex mechanisms of cell proliferation that also include precise integration of one or more functions of the JNK pathway. Thus, liver regenerative process in a mouse model provides an analytical platform to analyze the modulatory effects of Gadd45 β or absence thereof on the JNK pathway.
11. FIGS. 1A and 1B of Exhibit A demonstrate that, in the absence of Gadd45 β , following hepatectomy, the regenerating liver undergoes augmented apoptotic cell death (programmed cell death), instead of proliferation as in a normal liver. Augmented programmed cell death is shown by increased transaminase levels in the sera of Gadd45 β knock-out mice (ALT and AST levels) and by increased necrotic index in liver sections, 48 hours after surgery (FIG. 1C, Exhibit A).
12. FIG. 2A of Exhibit A demonstrates that hepatocytes from the regenerating livers of Gadd45 β knock-out mice do not respond to partial hepatectomy and fail to proliferate as illustrated by BrdU assays that measures DNA replication in the mouse hepatocytes. +/+ indicates wild-type liver and -/- indicates Gadd45 β knock-out liver. BrdU assays were performed at time zero and 48 hours after surgery.

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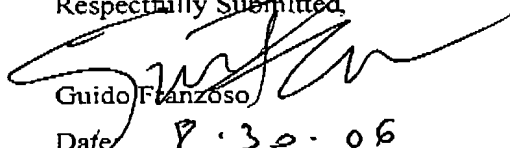
13. FIGS. 2B-2C of Exhibit A further demonstrate that livers of Gadd45 β knock-out mice undergo apoptotic cell death as illustrated by TUNEL assays on liver sections and electron microscopy (2B and 2C). TUNEL assays identified stained or positive nucleic undergoing cell death. +/+ indicates wild-type liver and -/- indicates Gadd45 β knock-out liver. TUNEL assays were performed at time zero and 48 hours after surgery.
14. In summary, data from FIGS. 2A-2C of Exhibit A offer proof that the absence of functional Gadd45 β in Gadd45 β knock-out mice results in increased cell death in the regenerating livers of mice.
15. Ablation of Gadd45 β in Gadd45 β knock-out mice also resulted in hyper activation of JNK activity (FIGS. 3A-3B, Exhibit A). Ablation of Gadd45 β also led to an increased activation of MKK7 (one of the direct targets of Gadd45 β). Other MAPK pathways, including the ERK and p38 pathways, do not appear to be affected by the absence of presence of Gadd45 β . The blots shown on FIGS. 3A-3B of Exhibit are western blots with anti-phospho-specific antibodies that show kinetics of kinase activity (kinase assay).
16. To further demonstrate that JNK activation leads to cell death in mice following hepatectomy, Gadd45 β knock-out mice was crossed with a JNK2 knock-out mice, resulting in Gadd45 β — JNK2 double knock-out mice. The regenerative and necrotic defects of the absence of Gadd45 β in Gadd45 β knock-out mice are corrected (FIGS. 4A-4C, Exhibit A) as shown by the transaminase levels in the sera and the proliferative index (gWT-wild type; gKO- Gadd45 β knock-out; j2WT-JNK2 wild type; j2KO-JNK2 knock-out).

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17. Togo et al., (2004) showed that Gadd45 β is induced in the liver following partial hepatectomy (Togo S, Makino H, Kobayashi T, Morita T, Shimizu T, Kubota T, Ichikawa Y, Ishikawa T, Okazaki Y, Hayashizaki Y, Shimada H. Mechanism of liver regeneration after partial hepatectomy using mouse cDNA microarray. *J Hepatol.* 2004 Mar;40(3):464-71). We show in Exhibit A that Gadd45 β induction is required for antagonism (suppression) of cell death (both necrosis and apoptosis) and for inducing cell proliferation in liver following partial hepatectomy.
18. Thus, the data from Gadd45 β knock-out mice model and JNK2 knock-out mice model provide substantial evidence that Gadd45 β is a modulator of JNK pathway in regulating cell death *in vivo*. The mouse model further supports the results obtained earlier from cell culture and *in vitro* studies in the specification that demonstrated direct Gadd45 β -JNKK2 interaction and modulation of JNK pathway.
19. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully Submitted,


Guido FranzosoDate: 8.30.06

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